

## Virus excretion in smallpox \*

### 2. Excretion in the throats of household contacts

J. K. SARKAR,<sup>1</sup> A. C. MITRA,<sup>2</sup> M. K. MUKHERJEE,<sup>3</sup> & S. K. DE <sup>4</sup>

*Throat swabs of 34 of 328 family contacts of 52 smallpox cases, examined 4-8 days after the onset of the disease in the family, were positive for variola virus. The log titre of virus per swab ranged from 2 to 3.95. A higher proportion of unvaccinated than of vaccinated contacts excreted the virus. Only 4 of the virus-positive contacts developed clinical smallpox; this occurred 5-7 days after their swabs were examined. Excretion of virus in the throats of these contacts, a few of whom were in the incubation period of the disease, suggests the possibility that they could have spread the infection. This possibility, if kept in mind, may help in tracing the source of infection or in determining the incubation period in a few instances when difficulty is experienced.*

Smallpox is transferred direct from person to person in a continuing chain of transmission, and there is no recognized animal reservoir of the disease (WHO Expert Committee on Smallpox Eradication, 1972). It is generally believed that a patient is infectious to others from the time the disease is manifest. In tracing the source of an infection, therefore, it is usual to look for a smallpox patient from whom the new case might have caught the virus. The household contacts of smallpox cases are not usually thought of as possible sources of infection. In the study described here the virus content of the throats of household contacts was examined to determine if they could in any way be a potential threat in the spread of the disease.

#### MATERIALS AND METHODS

The word "contact" in this paper is used to denote a person belonging to the family of a virologically proved smallpox patient, living in intimate

contact with the case and often sleeping in the same room. The smallpox patients whose contacts were studied are designated "index cases"; they included patients admitted to the Infectious Disease Hospital, Calcutta, India, but the majority were cases encountered during our visit to the infected localities. The presence of a vaccination scar was taken as evidence of primary vaccination. No attempt was made to ascertain the date of primary vaccination or revaccination, as the information offered was unreliable. Patients with no vaccination scar and those vaccinated 0-7 days before our visit were considered as unvaccinated. However, all the contacts, whether unvaccinated or vaccinated, were given a vaccination after an occurrence of the disease in the family.

To denote the severity of the disease, the cases were divided broadly into "haemorrhagic", "confluent", and "discrete" according to the criteria described in part 1 of this article (Sarkar et al., 1973). The date of onset was taken as the day of onset of fever. Specimens were collected from contacts once, except on 4 occasions when the collection was repeated. Because only contacts available on the day of visit were examined, not all the contacts of the index cases were included in the study. If any of the contacts under study later developed smallpox, the cases were re-examined and further specimens were collected. The duration of contact was calculated from the day of onset of fever of the index cases.

\* Supported principally by a research grant from the World Health Organization.

<sup>1</sup> Professor of Virology and Officer-in-Charge, WHO Research Project on Smallpox, Department of Virology, School of Tropical Medicine, Calcutta, India.

<sup>2</sup> Research Officer, WHO Research Project on Smallpox, Department of Virology, School of Tropical Medicine, Calcutta, India.

<sup>3</sup> Demonstrator in Virology, School of Tropical Medicine, Calcutta, India.

<sup>4</sup> Assistant Director of Health Services (Smallpox), Government of West Bengal, Calcutta, India.

Table 1. Details of the virus-positive contacts of smallpox cases, the virus titre in their throats, and the clinical type of the index cases <sup>a</sup>

| Serial No. of contact | Age (years) | Primary vaccination | Serial No. and type of index case | Duration of contact (days) <sup>b</sup> | Log titre of virus per swab                                   | Remarks   |
|-----------------------|-------------|---------------------|-----------------------------------|---|---|---|
| 1                     | 5           | —                   | 1 (C) <sup>c</sup>                | 7                                       | 2.60  |   |
| 2                     | 19          | +                   | 1 (C)                             | 7                                       | 2.0   |   |
| 3                     | 7           | —                   | 1 (C)                             | 7                                       | 2.0   |   |
| 4                     | 11          | +                   | 2 (H) <sup>d</sup>                | 4                                       | 3.00  |   |
| 5                     | 24          | —                   | 2 (H)                             | 4                                       | 2.44  |   |
| 6                     | 58          | —                   | 3 (C)                             | 5                                       | 2.44  |   |
| 7                     | 62          | +                   | 3 (C)                             | 5                                       | 2.00  |   |
| 8                     | 17          | +                   | 4 (C)                             | 4                                       | 2.09  |   |
| 9                     | 11          | +                   | 5 (C)                             | 4                                       | 2.0   |   |
| 10                    | 9           | +                   | 5 (C)                             | 4                                       | 2.44  |   |
| 11                    | 5           | —                   | 6 (C)                             | 4                                       | 2.09  |   |
| 12                    | 3           | —                   | 7 (C)                             | 4                                       | 2.0   |   |
| 13                    | 12          | +                   | 8 (C)                             | 4                                       | 2.44  |   |
| 14                    | 14          | +                   | 8 (C)                             | 4                                       | 2.0   |   |
| 15                    | 7           | +                   | 9 (C)                             | 5                                       | 2.0   |   |
| 16                    | 9           | +                   | 9 (C)                             | 5                                       | 2.0   |   |
| 17                    | 52          | —                   | 10 (C)                            | 7                                       | 3.0   |   |
| 18                    | 46          | +                   | 10 (C)                            | 7                                       | 3.0   |   |
| 19                    | 45          | +                   | 11 (C)                            | 7                                       | 2.0   | Developed smallpox 5 days after swabbing  |
| 20                    | 6           | —                   | 12 (C)                            | 6                                       | 2.44  |   |
| 21                    | 5           | +                   | 12 (C)                            | 6                                       | 3.00  | Developed smallpox 5 days after swabbing  |
| 22                    | 23          | +                   | 13 (C)                            | 6                                       | 3.00  |   |
| 23                    | 1 ½         | —                   | 13 (C)                            | 6                                       | 2.44  |   |
| 24                    | 10          | +                   | 14 (C)                            | 7                                       | 2.95  |   |
| 25                    | 5           | +                   | 14 (C)                            | 7                                       | 2.0   |   |
| 26                    | 55          | +                   | 15 (C)                            | 6                                       | 3.95 (on 6th day)<br>2.00 (on 11th day)<br>0.00 (on 17th day) | Developed smallpox 5 days after swabbing. Revaccinated on 1st day of swabbing.                  |
| 27                    | 14          | —                   | 16 (C)                            | 7                                       | 2.44  |   |
| 28                    | 36          | +                   | 17 (C)                            | 4                                       | 2.00  |   |
| 29                    | 5 months    | —                   | 17 (C)                            | 4                                       | 3.00 (on 4th day)<br>2.00 (on 11th day)                       | Developed smallpox 7 days after 1st swabbing. Given primary vaccination on 1st day of swabbing. |
| 30                    | 1 ½         | —                   | 18 (C)                            | 7                                       | 2.0   |   |
| 31                    | 6           | —                   | 19 (C)                            | 5                                       | 2.44  |   |
| 32                    | 10          | +                   | 20 (C)                            | 8                                       | 2.0   |   |
| 33                    | 12          | —                   | 21 (H)                            | 7                                       | 3.44<br>1.00 (on 13th day)                                    |   |
| 34                    | 37          | +                   | 22 (C)                            | 8                                       | 2.0   |   |

<sup>a</sup> All contacts were vaccinated after the index cases were detected.

<sup>b</sup> From the onset of fever in the index case.

<sup>c</sup> C = confluent.

<sup>d</sup> H = haemorrhagic.

Before contacts' throats were swabbed, the cotton swabs were soaked in Hanks' basal salt solution (BSS) containing 0.5% bovine albumin and antibiotics (penicillin and streptomycin). The specimens were immediately placed in ice containers and brought to the laboratory. The swabs were dipped in 1 ml of Hanks' BSS and squeezed; the fluid was then preserved at  $-20^{\circ}\text{C}$  until inoculation. Egg inoculation and pock counts were performed as reported previously (Sarkar & Mitra, 1967). Identifications of variola virus were based on the characteristics of growth on egg chorioallantoic membrane (CAM).

#### RESULTS

Altogether, 328 contacts of 52 index cases were examined. Variola virus was present in the throats of 34, while the rest were negative. Data on the positive contacts and the log titres of virus in their throats are given in Table 1. There were 2 haemorrhagic and 20 confluent cases among the index patients and the duration of contact with them varied from 4 to 8 days. Eighteen of the contacts were vaccinated and 16 were unvaccinated. Log virus titre in the throat ranged from 2 to 3.95 per swab. In 4 contacts (serial Nos. 21, 26, 29, and 33) the examination was repeated and virus titres were found to fall gradually. Four of the contacts (serial Nos. 19, 21, 26, and 29) developed smallpox; 3 of them had been vaccinated previously and one (serial No. 29) was vaccinated during the incubation period.

The frequency of isolation of variola virus by age group from vaccinated and unvaccinated contacts is shown in Table 2, while Table 3 gives the frequency of isolation of virus among contacts exposed to haemorrhagic, confluent, and discrete cases of smallpox.

#### DISCUSSION

In this study 34 (10.36%) of the 328 contacts examined were found to have variola virus in their throats, but only 4 (8.8%) of these 34 later developed the disease. Thus 30 contacts out of 328 were capable of excreting virus at some stage, although they never developed smallpox. Unfortunately it was not possible to perform serial throat cultures, and so the duration of these contacts' infectivity could not be assessed. For the same reason it is not known if any of the negative contacts excreted virus on any day before or after our visit. One contact (serial No. 33) who did not develop smallpox yielded throat swabs positive for virus on the 7th and 13th days of illness

of his index case. It may be assumed that in the intervening period his throat carried the virus, although the virus titre was lower in the second sample.

The quantum of virus excreted by a person has a definite bearing on infectivity. In all the contacts virus titre was more than  $10^2$  per swab, and in a number it was above  $10^3$ . These concentrations of virus in the throat are hardly negligible, as similar concentrations were found in the throats of a number of smallpox cases (Sarkar et al., 1973). Because serial throat swabs were not taken we cannot say whether the virus titre in contacts' throats was ever higher than on the day of the single visit. Unfortunately we do not yet know what is the minimum number of virus particles that must be implanted in the throat of a susceptible individual to produce disease in him.

The potential ability of contacts to spread smallpox thus requires serious consideration, particularly since little restriction is put on their movements and any spread of infection by them, should it occur, will remain unnoticed. When searching for a source of infection epidemiologists are sometimes unable to establish that a new case has been in direct contact with a smallpox patient. In such a situation, the possible role of contacts in spreading the infection may merit consideration.

There is at present no epidemiological support for the concept of spread of smallpox by household contacts. But when the origin of a new case is traced to a visit to the house of an established case, it is difficult to say categorically that the visitor caught the infection from the case and not from a virus-positive household contact. Indeed, there is every chance of his coming into closer contact with members of the household than with the actual patient.

Four of the contacts in this study (Nos. 19, 21, 26, and 29 in Table 1) developed smallpox 5-6 days after virus was detected in their throats. This raises the possibility of the spread of infection by a person while in the incubation period. The titre of virus in the 4 cases, particularly case 26, was fairly high, indicating that there was a fair chance of their spreading the disease to others. This finding, although not contradictory, is not consistent with the failure of McCallum et al. (1950) and Downie & McCarthy (1954) to isolate virus from the mouths of smallpox patients before rash appeared. The failure to isolate virus from the mouth washings of 5 smallpox cases during the first 2 days of the disease (Downie et al., 1961) might, as the authors pointed out, be due to

Table 2. Frequency of isolation of variola virus, by age group, from vaccinated and unvaccinated household contacts of smallpox cases

| Age (years) | Vaccinated contacts             |                         |   | Unvaccinated contacts           |                         |   |
|-------------|---------------------------------|-------------------------|---|---------------------------------|-------------------------|---|
|             | No. from whom cultures obtained | No. with smallpox virus | No. who later developed clinical smallpox | No. from whom cultures obtained | No. with smallpox virus | No. who later developed clinical smallpox |
| < 1         | 14                              | 0                       | 0   | 17                              | 1 (5.8 %)               | 1   |
| 1-4         | 34                              | 0                       | 0   | 19                              | 3 (15.7 %)              | 0   |
| 5-14        | 45                              | 11 (24.4 %)             | 1   | 8                               | 7 (87.5 %)              | 0   |
| > 15        | 176                             | 7 (3.9 %)               | 2   | 15                              | 5 (33.3 %)              | 0   |
| totals      | 269                             | 18 (6.7 %)              | 3   | 59                              | 16 (27.1 %)             | 1   |

the fact that only a very small portion of the mouth washings was examined. It is difficult to reconcile the present findings with actual field experience, which indicates that smallpox transmission by droplets occurs mainly during the first week of the manifested disease (Rao et al., 1968). It is noteworthy that in cases 26, 29, and 33 the titre of virus in the throat fell to a certain extent after the disease became manifest. All 3 patients were vaccinated or revaccinated during the incubation period, and it might be that vaccination played a part in the subsequent fall in virus titre. Two of the previously vaccinated contacts (Nos. 19 and 26) who developed smallpox were aged 45 and 55 years; it can be presumed that they had been vaccinated many years earlier. Case No. 21, aged 5 years, might have had his primary vaccination more than 4 years previously and case 29, a 5-month-old infant, was not vaccinated until the index case occurred in the family. Table 1 shows that 16 other unvaccinated contacts harbouring

virus in their throats did not develop smallpox. In some of them, vaccination after exposure to the virus may have prevented the disease, and in others previous subclinical infection may have produced a level of immunity sufficient to arrest the onset of the disease process. Unfortunately the sera of the latter cases were not examined for antibody to test this hypothesis.

In some patients the incubation period of the disease, calculated from the case history and apparent source of infection, does not fit the accepted incubation period. Here it is worth quoting the report of the United Kingdom Ministry of Health (1963) on the smallpox outbreak in Great Britain in 1961-1962: "In other instances what might normally be regarded as the conjectured time of infection proves to fall when the patient and his supposed source of infection were not in contact. One has then to postulate a longer or shorter incubation period in order to continue to assume the relationship between

Table 3. Frequency of isolation of variola virus from household contacts exposed to haemorrhagic, confluent, and discrete cases of smallpox

| Type of index case | Vaccinated contacts             |                         |   | Unvaccinated contacts           |                         |   |
|--------------------|---------------------------------|-------------------------|---|---------------------------------|-------------------------|---|
|                    | No. from whom cultures obtained | No. with smallpox virus | No. who later developed clinical smallpox | No. from whom cultures obtained | No. with smallpox virus | No. who later developed clinical smallpox |
| Haemorrhagic       | 18                              | 1 (5.5 %)               | 0   | 4                               | 2 (50 %)                | 0   |
| Confluent          | 216                             | 17 (7.9 %)              | 3   | 40                              | 14 (35 %)               | 1   |
| Discrete           | 35                              | 0                       | 0   | 15                              | 0                       | 0   |
| totals             | 269                             | 18 (6.7 %)              | 3   | 59                              | 16 (27.1 %)             | 1   |

the two cases; or one must look elsewhere for a source on this occasion. Such a source might be a 'missed case', and this suggests that persons other than contacts under observation have been placed at risk. The absence of other discovered cases in the general population weakens the 'missed case' hypothesis and tends to throw one back on the assumption that, in these instances, the incubation period was in fact longer or shorter than usual." The recognition that contacts of smallpox cases or patients during the incubation period can excrete the virus may explain some of the discrepancies observed in the incubation period. If a patient picks up the infection from a case before the disease is manifest, the incubation period will appear to be shorter than usual, while if he is infected by a contact of a smallpox case, the incubation period might appear to be longer.

The data in Table 1 indicate that the age and vaccination status of contacts did not materially affect the virus content of their throats. Although there were only 2 haemorrhagic cases among the 22 index cases, their contacts had the same virus titre in their throats as the contacts of the 20 confluent cases. It is apparent from Table 3 that the

haemorrhagic cases did not contribute more virus-positive contacts than the confluent cases. Virus-negative contacts were also associated with all 52 index cases studied. In other words, individual index cases had both virus-positive and virus-negative contacts.

Regarding age or period of contact with the index cases, the virus-negative contacts did not differ materially from the virus-positive contacts. However, the two groups differed considerably in respect of vaccination status; 15.30% of the virus-negative contacts were unvaccinated, as against 47.05% in the virus-positive group. Of the total of 328 contacts studied, 16 of 59 (27.1%) unvaccinated contacts, but only 18 of 269 (6.7%) vaccinated contacts, were virus-positive. This shows that previous vaccination had a marked influence on virus excretion in contacts' throats (Tables 2 and 3).

The fact that only 10.36% of contacts were virus-positive indicates that their potential for spreading the infection is much less than that of smallpox patients, all of whom are capable of excreting virus in the throat (often at a higher titre) or the scabs, and many of whom excrete in the urine and conjunctiva (Sarkar et al., 1973).

## RÉSUMÉ

### EXCRÉTION DE VIRUS AU COURS DE LA VARIOLE: 2. EXCRÉTION PAR LA GORGE CHEZ DES CONTACTS FAMILIAUX

On a effectué des prélèvements de sécrétions pharyngées chez 328 contacts appartenant à l'entourage familial de 52 varioleux 4 à 8 jours après la découverte du premier cas. Chez 34 d'entre eux (10,36%), le virus était présent à des titres variant en valeur logarithmique de 2 à 3,95 selon le prélèvement. Quatre de ces sujets positifs ont été atteints de variole 5-7 jours après le prélèvement, tandis qu'aucun cas ne s'est déclaré chez les contacts non excréteurs de virus. On comptait 47% de sujets non vaccinés antérieurement parmi les excréteurs de virus et 15,3% parmi les non excréteurs, les deux groupes étant par ailleurs comparables sous le rapport de l'âge et de la durée du contact avec le cas princeps. Une

vaccination antérieure a fortement influencé le taux d'excrétion de virus parmi les contacts: 27,1% des contacts non vaccinés et 6,7% des vaccinés étaient positifs. Tous les contacts, déjà vaccinés ou non, ont reçu la vaccination antivariolique au moment du prélèvement ou dans les jours qui l'ont précédé.

La présence du virus variolique dans les sécrétions pharyngées de contacts, dont certains étaient en période d'incubation de la maladie, donne à penser qu'ils ont pu transmettre l'infection. On doit tenir compte de cette possibilité lorsqu'il s'agit de déterminer la source de l'infection ou la durée de la période d'incubation dans des cas d'interprétation malaisée.

## REFERENCES

- Downie, A. W. & McCarthy, K. (1954) *Pathogenesis of variola*. In: Hartman, W. F. et al., ed., *The dynamics of virus and rickettsial infections*, New York, Blakiston, pp. 194-205
- Downie, A. W. et al. (1961) *Bull. Wld Hlth Org.*, **25**, 49-53
- MacCallum, F. O. et al. (1950) *Lancet*, **259**, 514-517
- Rao, A. R. et al. (1968) *Indian J. med. Res.*, **56**, 1826-1854
- Sarkar, J. K. & Mitra, A. C. (1967) *Indian J. med. Res.*, **55**, 13-20
- Sarkar, J. K. et al. (1973) *Bull. Wld Hlth Org.*, **48**, 517
- United Kingdom, Ministry of Health (1963) *Smallpox 1961-62*, London, Her Majesty's Stationery Office (*Reports on Public Health and Medical Subjects*, No. 109)
- WHO Expert Committee on Smallpox Eradication (1972) *Wld Hlth Org. techn. Rep. Ser.*, No. 493